PHYSIOLOGICAL STUDIES ON THE VITAMIN C CONTENT OF MARINE ALGAE

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By

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Introduction

Within the last fifteen years, several scientists have conducted research on the vitamin C content of marine algae (1, 2, 3, 4, 5, 6). Among these, Norris, Simeon and Williams (2) were the first to recognize the close relationship between the depth of water and the vitamin C content of marine algae. They found that the vitamin C content of algae collected from shallow water was greater than that from deep water. Koizumi and Kakukawa (5) have also reported that the vitamin C content of marine algae decreases with the depth of water; that in its diurnal changes, the vitamin C content was found to increase in the daytime and to decrease at night; and that the increase of the vitamin C content is accelerated by an increase in the intensity of light, as with higher green plants.

This paper deals with the results of experiments conducted by the writer, (1) on the seasonal variations in the vitamin C content of certain marine algae in relation to the other chemical components of these same algae; and also (2) on the effects of temperature, hydrogen-ion concentration and chlorinity upon the increase of vitamin C in these plants.

Materials and methods

In his experiments the writer used two species of green algae, *Ulva pertusa* Kjellman and *Enteromorpha* sp, and one species of red algae, *Gracilaria confervoides.* These were collected from Hakata Bay in Fukuoka Prefecture.

Vitamin C content was determined by Fujita's method of direct titration of ascorbic acid by 2, 6-dichlorophenolindophenol (7). The experiments were performed under the following conditions: (1) the materials collected were immersed as soon as possible in filtered sea-water in an aquarium, and allowed to stand at room temperature for twenty-four hours, shaded

from direct sunlight; (2) analyses were always conducted at a definite time of day, between one and two o'clock in the afternoon, in order to eliminate any discrepancy in the diurnal changes in vitamin C content. Analyses of other general components, except for carbohydrate, were made by the usual methods. The carbohydrate content was determined as glucose by the Bertrand method after being hydrolysed with 2n-HCl for two hours in a boiling water bath.

Results

J. *Seasonal variations in vitamin C content.*

Observations of seasonal variations in the vitamin C content of marine algae were conducted over a period of approximately six months, from December 10, 1946 to June 7, 1947. The results are shown in Table I and Figure 1.

Table I

Seasonal variation in vitamin C and general components of marine algae (Contents of protein, carbohydrate, ash and vitamin C are expressed in percentage of dry weight.)

Date	1946 Dec. 10	Jan. 8	Jan. 28	1947 March 4	April 4	May 7	June 7
Water Temperature (°C)	8.0	6.0	2.5	4.0	13.0	18.0	—
Ulva pertusa Kjellman							
Moisture (%)	80 50	80.51	79.32	77.54	82.63	81.88	78.99
Protein (%)	25.75	28.98	28.48	33.01	30.05	20.08	9.12
Carbohydrate (%)	17.51	17.58	18.16	18.83	19.35	21.86	41.18
Ash (%)	24.52	17.71	19.06	16.73	19.58	22.47	22.25
Vitamin C (mg%)	83.51	241.23	193.50	204.44	171.06	173.02	30.34
Enteromorpha sp.							
Moisture (%)	78.04	84.24	88.73	83.81	83.05	85.86	85.36
Protein (%)	19.30	26.30	28.98	23.50	17.82	17.82	15.43
Carbohydrate (%)	18.02	22.15	25.44	31.07	37.80	26 52	21.25
Ash (%)	30.97	25.00	17.57	20.12	20.61	15.25	26.52
Vitamin C (mg%)	90.03	171.28	238.85	227.70	195.02	131.22	64.24
Gracilaria confervoides							
Moisture (%)	85.45	83.42	83.06	81.78	90.65	90.75	85.14
Protein (%)	26.30	30.05	27.58	27.56	22.58	18.53	10.05
Carbohydrate (%)	28.00	30.08	31.62	34.28	30.75	33.06	36.51
Ash (%)	19.65	24.11	22.58	18.40	19.18	22.01	25.12
Vitamin C (mg%)	73.52	95.98	158.52	148.63	149.80	167.62	16.38

Figure 1 shows that the vitamin C content of the *Ulva pertusa* reached a maximum of 241.23 milligrams per cent at the beginning of January. It then decreased gradually until May, when it fell off rapidly to 30.34 milligrams per cent. In the case of the *Enteromorpha* sp., it reached

a maximum of 238.85 milligrams per cent at the end of January, gradually declined until the beginning of April, and finally dropped rapidly to 64.24 milligrams per cent. The *Gracilaria confervoides*, on the other hand, did not distinctly show any maximum. It steadily increased until the end of January and then stayed at a level of from 148.63 to 167.62 milligrams per cent, until it fell abruptly in the middle of May.

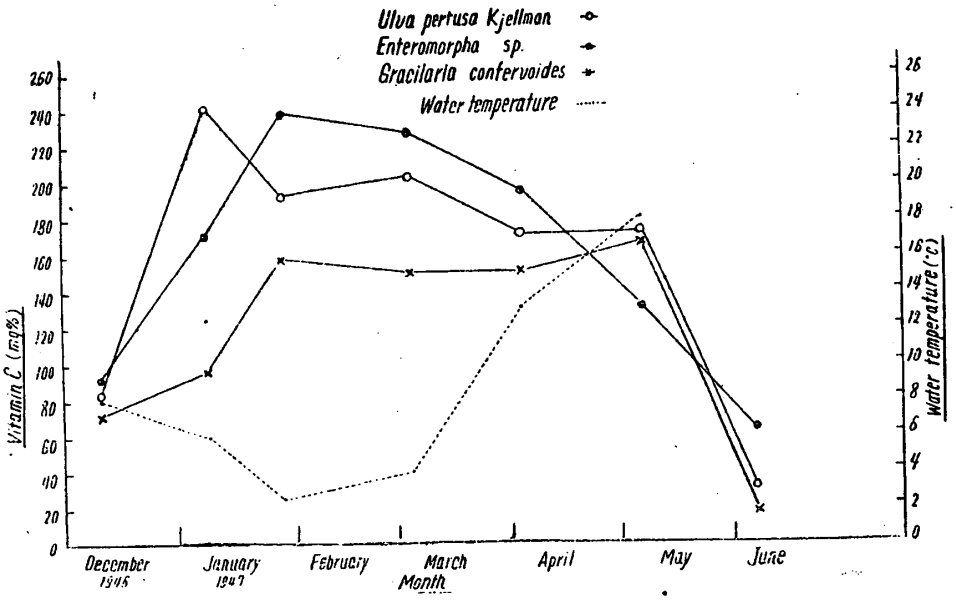

Fig. 1. Seasonal variation in vitamin C content of marine algae.

It was generally observed, though this was not so evident in the *Gracilaria confervoides*, that the vitamin C content of the marine algae showed an inverse relation to the temperature of the water. Specifically, the vitamin C content was greatest when the temperature of the water was lowest. It is particularly significant to note that the season of the maximum content of vitamin C in the *Enteromorpha* sp. (the end of January) coincided with that of the minimum temperature of the water.

As for seasonal variations in the other general components of the algae, only the protein content was observed to vary and this variation showed a faint relation to that of the vitamin C content. No such tendency was noticed in the other components (Figure 2). This relationship between the vitamin C and protein content of the algae is considered to be attributable to their common role in the physiological life of these algae. Furthermore, from the coincidence in the seasons of the minimum contents, it is suggested that both vitamin C and protein are employed in later spring and early summer in the fructification of the algae.

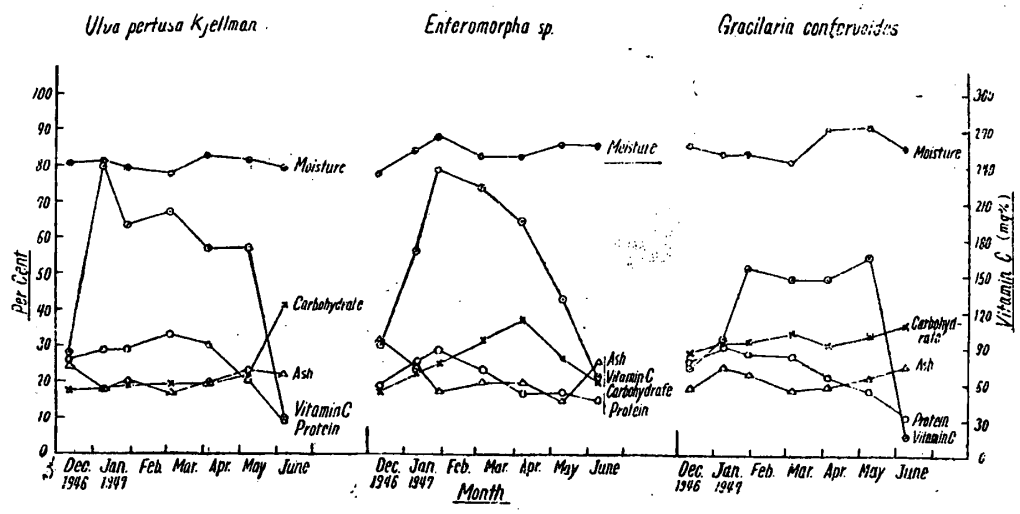

Fig. 2. Seasonal variation in general components and vitamin C of marine algae.

II. *The effects of temperature, hydrogen-ion concentration and chlorinity on vitamin C content.*

The effects of temperature, hydrogen-ion concentration and chlorinity on vitamin C content were determined by the following procedure:

a) The effect of temperature was determined by immersing the specimens for three hours in sea-water of varying temperatures. This experiment was conducted for a period of three days, from 13 to 15 January, 1947.

b) The effect of hydrogen-ion concentration was determined by immersing the specimens in sea-water at room temperature, with the hydrogen-ion concentration varied (at regular intervals) by the addition of NaOH or HCl, for a period of seven days, from 23 to 30 December, 1946.

c) The effect of chlorinity was determined by immersing the specimens in sea-water at room temperature, with the chlorinity varied (at regular intervals) by the dilution of concentrated sea-water for a period of three days, from 13 to 16 December, 1946.

The results of these experiments are shown in Figure 3.

The above experiments indicate that the temperature of sea-water has a marked effect upon the vitamin C content of marine algae, just as temperature is known to effect the vitamin C content of higher green plants (8. 9). When kept for three hours at low (below 10°C) temperatures, or at high (above 30°C) temperatures, their physiological activity was retarded and their production of vitamin C was slight in relation to their production of vitamin C at moderate (about 20°C) temperature.

The effect upon vitamin C content of hydrogen-ion concentration differed

in the case of each species of algae. Both *Ulva pertusa* and *Enteromorpha* sp. showed a greater sensibility to changes in hydrogen-ion concentration; and in either acid or alkaline sides, beyond a narrow range of from pH 7.8 to 8.2 in *Enteromorpha* sp. and pH 8.0 to 8.5 in *Ulva pertusa*, the production of vitamin C was invariably low. *Gracilaria confervoides*, on the other hand, showed no appreciable reaction to variations in hydrogen-ion concentration, and the production of vitamin C in this species remained within the broad range of from pH 7.5 to 9.5.

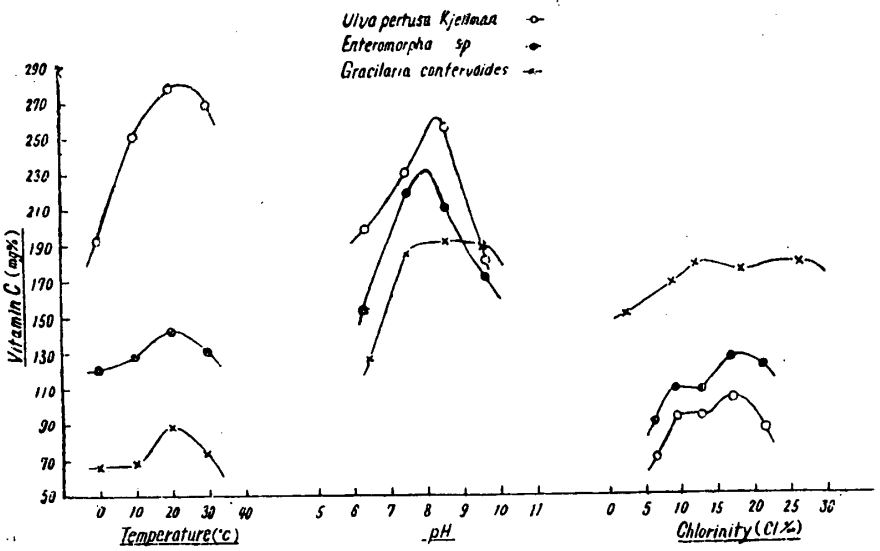

Fig. 3. The influence of temperature, hydrogen-ion concentration and chlorinity on vitamin C content of marine algae.

Vitamin C production in *Gracilaria confervoides* was high within a broad range of chlorinity of from 13 to 27 per mil. In the other two species, vitamin C production was relatively high only within a narrow range of 15 to 20 per mil, outside this range, it was markedly low. This circumstance is probably because of the structure of the protective tissues in *Gracilaria confervoides* which are thicker and denser than those of *Enteromorpha* sp. and *Ulva pertusa*.

A general conclusion can be drawn from the above that, like the vitamin C content of higher green plants, the vitamin C content of some marine algae is influenced by their physical and chemical environmental conditions.

Summary

1. Observations were made on the seasonal variations in the vitamin C content of certain marine algae in relation to the other chemical components

of these same algae; and research was conducted on the effects of temperature, hydrogen-ion concentration and chlorinity upon the increase of vitamin C in these plants.

2. The vitamin C content of *Ulva pertusa* kjellman reached a maximum of 241.23 milligrams per cent at the beginning of January, and of *Enteromorpha* sp. it reached a maximum of 238.85 milligrams per cent at the end of the same month. *Gracilaria confervoides* showed no distinct maximum, but stayed at a level of from 148.63 to 167.62 milligrams per cent for a long period, from the end of January to the beginning of May.

3. Of the other general components of these algae, only the protein content was observed to undergo seasonal variations and these showed a faint relation to variations in the vitamin C content.

4. The vitamin C content of some marine algae is influenced by physical and chemical environmental conditions in a manner similar to that of these influences on the vitamin C content of higher green plants.

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